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14. ABSTRACT

The metabolic consequences of obesity may be critical in the development of ovarian cancer (OC), resulting in biologically different cancers than those that arise in leaner women. This may occur through aberrant modulation of mTOR signaling, given that alterations in this pathway are common in both obesity and OC. Thus, obese OC patients may derive increased benefit from chemotherapeutic agents related to inhibition of this pathway, such as mTOR inhibitors (everolimus) or metformin. We have demonstrated that the obese state can promote tumor progression in the KpB mouse model of OC. The ovarian tumors that arose in the obese mice were genomically and metabolically different from those that arose in non-obese mice. Metformin was found to be more efficacious in the obese versus non-obese KpB mice, suggesting that obesity may be a biomarker for response to this agent. For our in vitro studies, metformin and everolimus were found to be more effective in the inhibition of proliferation and induction of apoptosis under low versus high glucose concentrations. We postulate that OC cells deprived of glucose have blunted proliferative capacity, rendering them more susceptible to metformin and everolimus, and that a high glucose environment may overall enhance proliferative capacity.

15. SUBJECT TERMS
Ovarian cancer, mTOR pathway, mTOR inhibitors, metformin, obesity, genomics, metabolomics

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INTRODUCTION

Obesity leads to elevated incidence and worse outcomes for ovarian cancer (OC) (1-18). We postulate that the metabolic consequences of obesity may be crucial in the development of ovarian cancer, resulting in biologically different cancers than those that arise in normal weight women. This may occur through aberrant modulation of mTOR signaling, given that alterations in this pathway are common in both disease processes. Thus, obese ovarian cancer patients may derive increased benefit from chemotherapeutic agents related to inhibition of this pathway, such as mTOR inhibitors or metformin. This proposal will address this question by investigating the impact of obesity on the proliferative and metabolic effects of mTOR inhibitor and metformin treatment in three model systems: *in vitro* using OC cell lines; *in vivo* using a novel serous ovarian tumor murine model; and in a pilot clinical trial. The role of obesity in OC initiation and promotion will be evaluated through comprehensive cross-species genomic and metabolomic analysis with the goal of identifying common genetic or metabolic biomarkers associated with obesity-driven cancers and differential response to treatment in the obese and non-obese state. If our hypothesis is true, optimization of OC treatment may need to encompass tumor characteristics as well as obesity status.

BODY

Task 1 (Aim 1): To assess the effect of the mammalian target of rapamycin (mTOR) inhibitor everolimus and metformin on key metabolic pathways in human ovarian cancer cell lines under high and low glucose conditions.

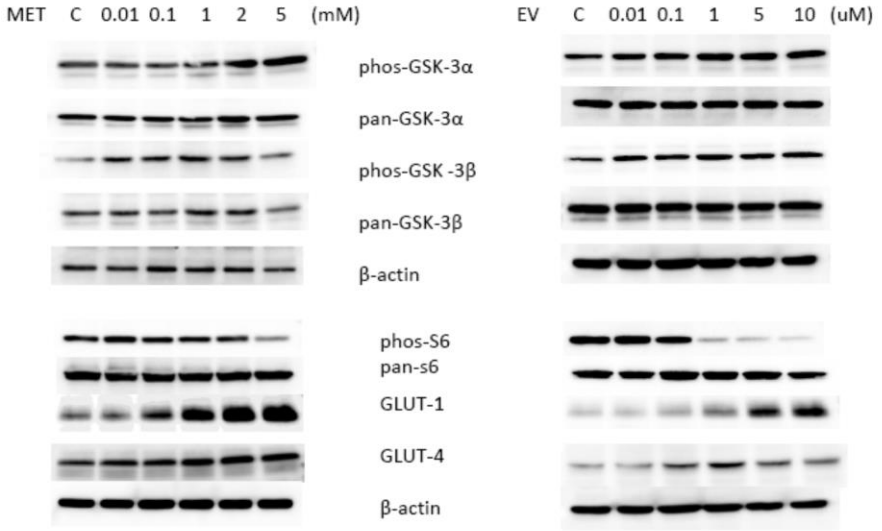

Figure 1. Metformin and everolimus increased phosphorylation of GSK-3β and GSK-3α and reduced phosphorylation of S6 expression in a dose dependent manner in the SKOV3 cell line after 24 hours of treatment, as assessed by Western immunoblotting analysis. In parallel, metformin and everolimus increased GLUT1 and GLUT4 expression.

Overweight and obese states may be linked to OC through nutrient-sensitive signaling cascades, such as the insulin/insulin growth factor (IGF) and PI3K/Akt/mTOR pathways (19-23). Hyperinsulinemia, insulin growth factor-1 (IGF-1) and IGF-1 receptor (IGF-1R) levels are important in OC development and progression in experimental and epidemiological studies (24-27). Signaling through IGF-1R leads to activation of the PI3K/Akt/mTOR pathway, and components of this pathway are often mutated, amplified or aberrantly expressed in OCs (28-33). Thus, mTOR inhibitors, such as everolimus, as a targeted therapy for OC are currently being actively investigated in Phase 1, 2 and 3 clinical trials (34-36).

Metformin is an anti-diabetic medication from the biguanide class that is widely used as the first line treatment of type 2 diabetes. Mounting epidemiological evidence suggests that metformin use lowers cancer risk and reduces cancer deaths among diabetic patients (37-39), including ovarian cancer (40-42). Metformin is believed to have both indirect and direct effects on tumor growth, and it is unknown which of these effects are most important for metformin's anti-tumorigenic benefits. Its indirect effects are likely to be due to inhibition of hepatic gluconeogenesis, resulting in an improvement in insulin sensitivity and a reduction in blood glucose and circulating insulin levels, which may lead to decreased growth factor-stimulated tumor growth. On a direct level, metformin may affect tumor growth by activation of adenosine monophosphate-activated protein kinase (AMPK), its intracellular target for anti-diabetic effects, which leads to the regulation of multiple downstream signaling pathways that control cellular proliferation, including inhibition of the mammalian target of rapamycin (mTOR) pathway.

Given that we have previously shown that metformin and mTOR inhibitors are potent inhibitors of OC cell proliferation (43, 44), we wanted to assess whether these agents also had an effect on glucose metabolism

in OC cell lines. Western immunoblotting analysis revealed that treatment with metformin and the mTOR inhibitor, everolimus, increased facilitative glucose transporter 1 and 4 (GLUT-1 and GLUT-4) expression and increased phosphorylation of glycogen synthase kinase-3 alpha and beta (GSK-α and GSK-β) (**Figure 1**). In parallel, metformin and everolimus inhibited the mTOR pathway, as evidenced by decreased phosphorylation of its downstream target, S6 (**Figure 1**). These results suggest that treatment with metformin and everolimus potentially drives glucose metabolism and uptake in OC cells, despite blunting proliferation.

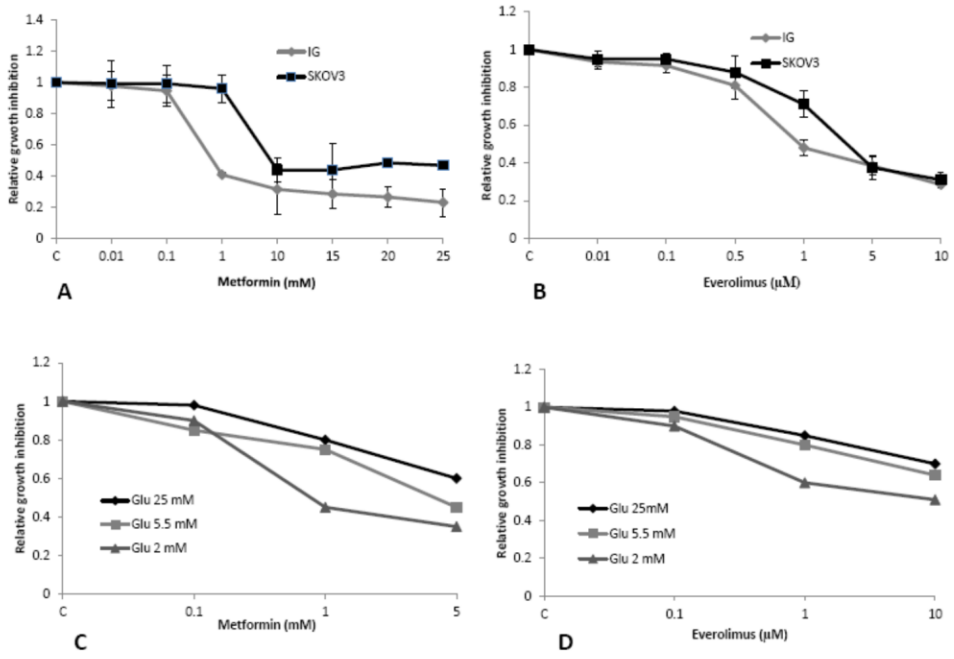

Figure 2. The SKOV3 and IGROV1 (IG) cell lines were treated with metformin and everolimus under low glucose (2 mM), normal glucose (5.5 mM) or high glucose (25 mM) conditions for 72 hours. Cell growth was determined by MTT assay. Metformin and everolimus inhibited proliferation in both cell lines under normal glucose conditions (**A and B**). Metformin and everolimus were found to be more effective in the inhibition of proliferation under low versus high glucose concentrations (**C and D**).

Subsequently, the effects of metformin and everolimus treatment on cell proliferation and apoptosis was assessed under normal, low and high glucose conditions in OC cell lines. Our goal was to mimic the obese-diabetic state *in vitro* through the use of high physiologic glucose. As expected, metformin and everolimus inhibited proliferation in both cell lines under normal glucose conditions (**Figure 2A and 2B**), through G1 cell cycle arrest (**Figure 3A and 3B**). Metformin and everolimus were found to be more effective in the inhibition of proliferation under low versus high glucose concentrations (**Figure 2C and 2D**), as also evidenced by enhanced G1 cell cycle arrest (**Figure 3C and 3D**). In addition, metformin and everolimus were found to be more effective in the induction of apoptosis under low versus high glucose concentrations (**Figure 4**). For all of these experiments,

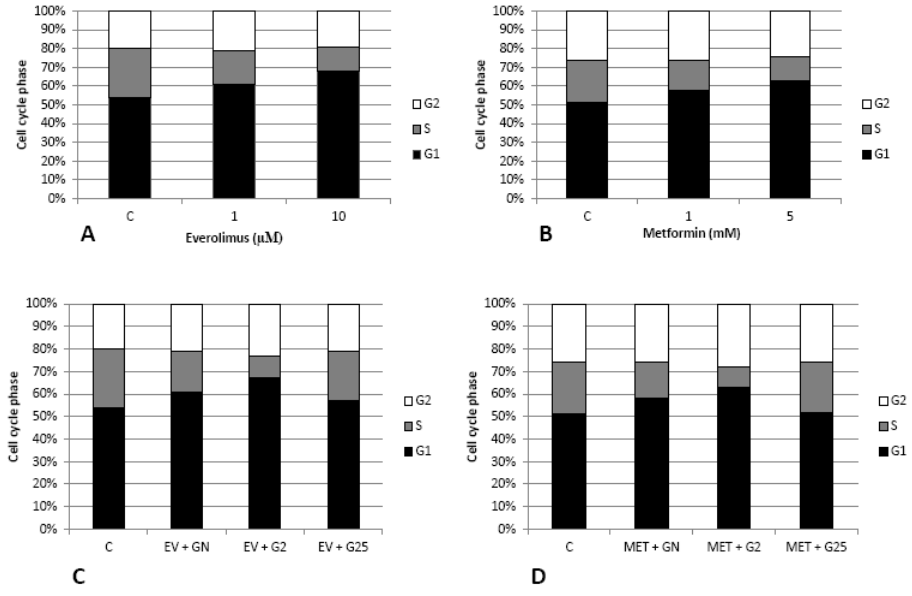

Figure 3. Metformin and everolimus induced G1 cell cycle arrest in the SKOV3 cell line (**A, B**). The SKOV3 cell line was treated with metformin (1 mM) or everolimus (1 uM) under low glucose (2 mM, G2), normal glucose (5.5 mM, GN) or high glucose (25 mM, G25) conditions for 24 hours (**C, D**). Metformin and everolimus were found to be more effective in G1 cell cycle arrest under low versus high glucose conditions. Cell cycle phase analysis was assayed by cellometer.

the effects of metformin and everolimus under normal glucose conditions was intermediary between low and high glucose levels. These results were opposite to what we had predicted; we had hypothesized that metformin and everolimus would be more effective under high glucose as opposed to low and normal glucose conditions.

Given these unexpected findings, we did examine the effects of differing glucose conditions (i.e. low, normal and high) alone on AMPK activation and mTOR pathway inhibition in the OC cells. As the concentration of glucose increased, we found that phosphorylation of AMPK decreased and phosphorylation of S6 increased, suggesting increased proliferative capacity and hyperactivation of the mTOR pathway with high physiologic glucose (**Figure 5**). Metformin and everolimus were effective under all glucose conditions in the OC cells; however, we observed heightened sensitivity under low versus high glucose conditions. Thus, we postulate that high glucose levels may override some of the anti-proliferative effects of both of these agents *in vitro*, and that cells exposed to low glucose may have blunted proliferative capacity and may be more inherently susceptible to metformin and everolimus. Further studies in the upcoming report period will focus on metabolic assays to evaluate the full effects of metformin and everolimus on glucose uptake and metabolism (glycolysis and oxidation) under the varying glucose conditions. In addition, Western immunoblotting will be performed to assess the expression of the downstream signaling targets of everolimus and metformin in OC cell lines under high and low physiologic glucose concentrations. To date, we have mainly focused on only two OC cell lines, but will expand this work to four cell lines to ensure the consistency of our findings. This work should provide a detailed examination of glucose metabolism along with proliferation to determine the interrelationship of everolimus and metformin treatment on cell metabolism and growth under varying metabolic environments such as high and low glucose conditions.

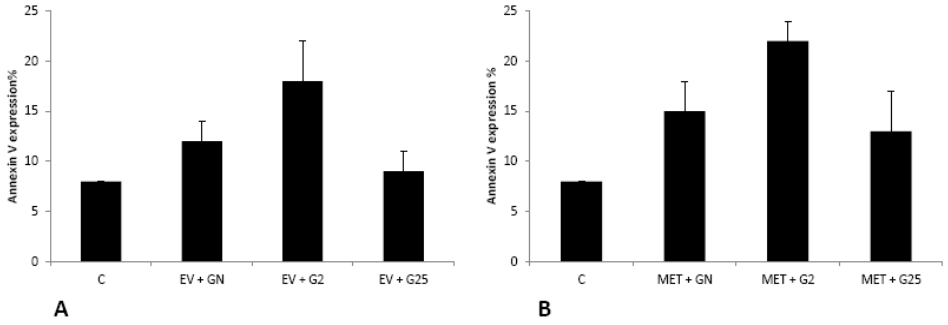

Figure 4. The SKOV3 cell line was treated with metformin (1 mM; B) or everolimus (1uM; A) under low glucose (2 mM; G2), normal (5.5 mM; GN) or high glucose conditions (25 mM; G25) for 24 hours. Metformin and everolimus were found to be more effective in the induction of apoptosis under low versus high glucose concentrations. Annexin V expression, a marker of apoptosis, was assessed by cellometer.

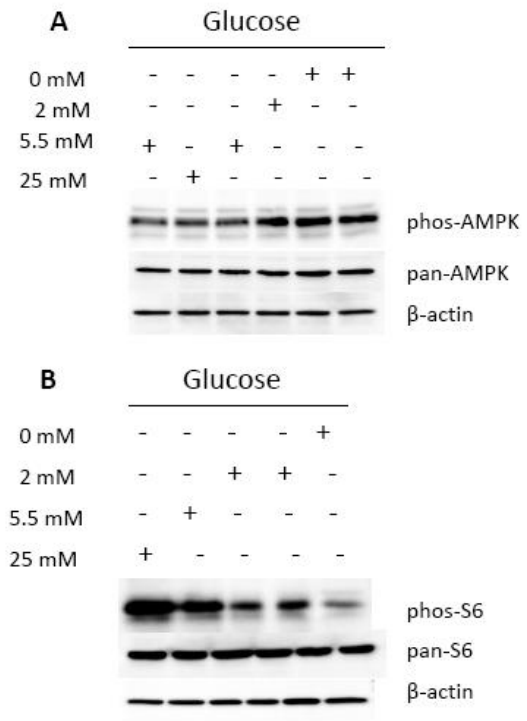

Figure 5. The SKOV3 cell line was exposed to different glucose concentrations for 24 hours. Phosphorylated (phos)-AMPK and S6 and pan-AMPK and S6 expression was determined by Western immunoblotting analysis. As the concentration of glucose increased, phosphorylation of AMPK decreased and phosphorylation of S6 increased.

Task 2 (Aim 2): To compare tumor latency, growth and response to treatment with the mTOR inhibitor everolimus and metformin in lean and obese K18-gT$_{121}^{+/-}$;p53$^{fl/fl}$;Brca1$^{fl/fl}$ (KpB) mice using genomics and metabolomics to identify pathways and biomarkers associated with therapeutic success.

We have previously described a unique serous ovarian cancer mouse model that specifically and somatically deletes the tumor suppressor genes, Brca1 and p53, and inactivates the retinoblastoma (Rb) proteins in adult ovarian surface epithelial cells (KpB mouse model) (45, 46). Subsequently, KpB mice were subjected to a 60% calories-derived from fat in a high fat diet (HFD) versus 10% calories from fat in a low fat diet (LFD) to induce diet-induced obesity (N=14/group) starting at 6 weeks of age and until sacrifice. After 8 months of exposure to the HFD or LFD, obese mice weighed significantly greater than non-obese mice (p=0.003, Table 1). There was no effect of HFD on non-fasted blood glucose levels or diabetes onset in KpB mice over the course of the diet (Table 1). Body composition was significantly altered in obese KpB mice compared to non-obese controls. Percent body fat was six-fold greater in obese mice (Table 1, p=0.0001), while percent lean mass increased by 25% (p=0.0006, Table 1). The ovarian tumors were tripled in size in the obese mice as compared to non-obese mice (mean size of 3.7 cm^2 versus 1.2 cm^2, **Figure 6**, p=0.0065). This suggests that obesity can promote tumor progression in this KpB mouse model of ovarian cancer.

Table 1. Diet-induced metabolic characteristics in non-obese and obese KpB mice.

	Non-Obese	Obese	p-value
Weight (gms)	31.14 ± 5.26	50.71 ± 16.73	p=0.0003
Glucose (mg/dl)	186.81 ± 26.99	214.38 ± 58.11	p=0.053
% Fat	3.28 ± 1.51	19.58 ± 7.88	p=0.00001
% Lean	22.89 ± 2.11	28.66 ± 5.24	p=0.0006
N=14 mice per group. Mean ± SD. % Fat or % lean = each mass/total body mass as measured by MRI.			

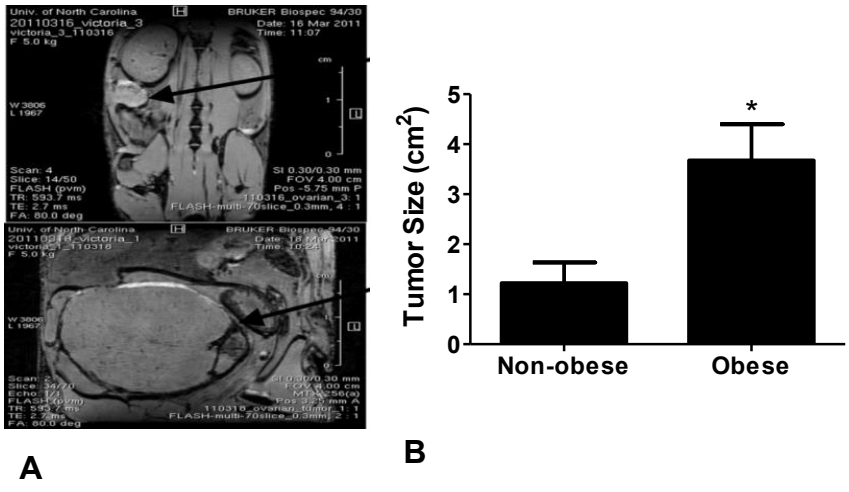

Figure 6. Obesity increases tumor size in KpB mice. KpB mice were fed low fat or high fat diets to induce obesity for 6 months during tumorigenesis. (A) MRI images of tumors (arrow) from non-obese (top image) and obese (bottom image) mice demonstrate representative tumors. (B) Comparison of tumor size from non-obese and obese mice (N=14). *p=0.0065.

Obesity was found to induce genomic differences between the obese and non-obese ovarian tumors. 439 genes were found to be significantly up-regulated (417 genes) or down-regulated (22 genes) in the ovarian tumors from obese KpB mice versus non-obese mice (FDR<0.2). **Figure 7** is a heat map of 131 genes up- and down-regulated at a FDR<0.1. Many metabolically relevant genes were significantly upregulated in the ovarian tumors from the obese versus non-obese mice, such as lipocalin (2.7 fold), fatty acid amide hydrolase (2.7 fold), fatty acid 2-hydroxylase (2.2 fold), glycerol-3-phosphate acyltransferase (1.5 fold), protein phosphatase (1.2 fold), AMP deaminase 3 (1.6 fold), and protein kinase C (1.7 fold). Arginase 1 was the most upregulated gene (7.3 fold) and plays a role in the urea cycle, tissue remodeling and inflammation. Other upregulated genes identified in the ovarian tumors from the obese mice were related to cell adhesion, including neurotrimin (2.2 fold) and desmoglein 1-alpha (2.0 fold). Increased expression of histone 1 (2.3 fold) and endothelin-1 (5.8 fold) were also associated with obesity in the KpB mouse model. Another gene upregulated 3 fold was ectonucleoside triphosphate diphosphohydrolase. Heparan sulfate (glucosamine) 3-O-sulfotransferase 1 was upregulated 6 fold and regulates heparan sulfate production which is involved in developmental processes, angiogenesis, blood coagulation and tumor metastasis. The serotonin transporter solute carrier family 6 member 4 (Slc6a4) was upregulated 5.4 fold by obesity. Important downregulated genes included spermidine synthase, an

enzyme in spermidine synthesis and thrombospondin 4, an extracellular glycoprotein known to have roles in cellular migration, adhesion, attachment and proliferation. In the ovarian tumors from the obese versus non-obese mice, DAVID functional annotation analysis revealed significant enrichment in “phospoholipid binding” (EASE score of 0.008), “regulation of apoptosis” (EASE score of 0.014), “lipid binding” (EASE score of 0.015), “endopeptidase activity” (EASE score of 0.03) and “cell-cell signaling” (EASE score of 0.44) for those identified genes.

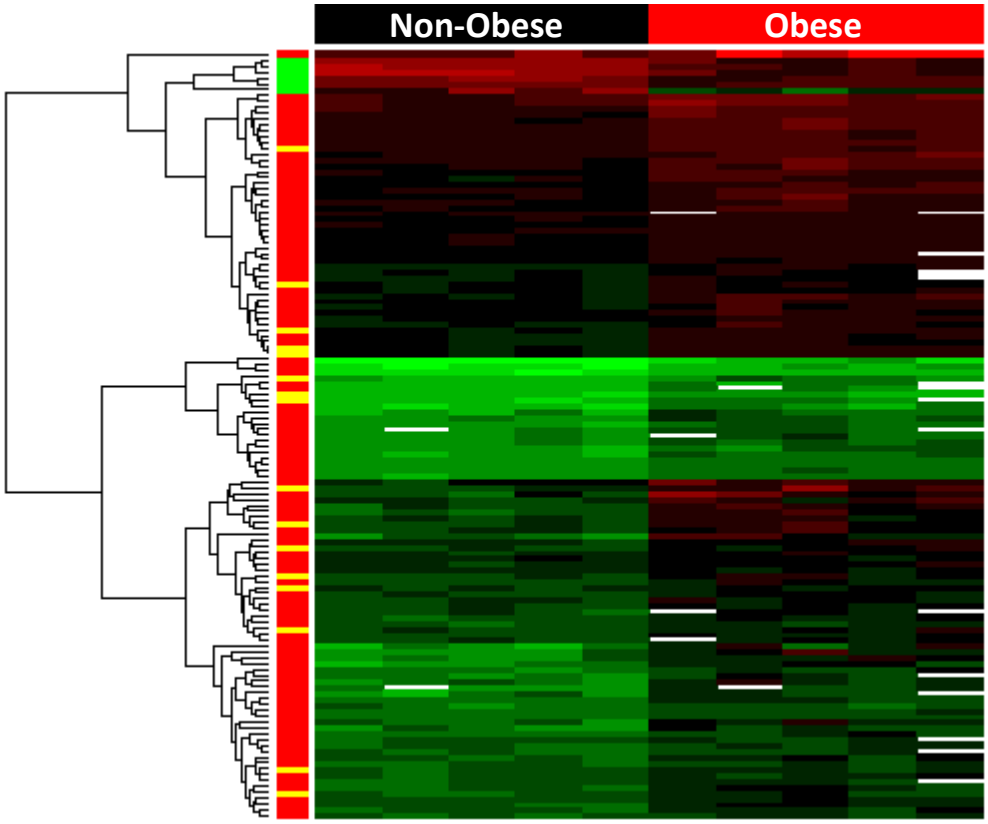

Figure 7. Genomic differences between ovarian tumors from obese versus non-obese KpB mice reveal alterations in metabolically relevant genes. Heat map representation of 131 genes found to be significantly up- or down-regulated in the ovarian tumors from the obese versus non-obese KpB mice (FDR<0.1). Many metabolically relevant genes, such as lipocalin, fatty acid amide hydrolase, ectonucleoside triphosphate diphosphohydrolase, fatty acid 2-hydroxylase, glycerol-3-phosphate acyltransferase, protein phosphatase, protein kinase C and AMP deaminase 3, were upregulated in obese tumors.

Metabolic differences were also found between the ovarian tumors from obese and non-obese KpB mice. Principle component analysis defined a clear separation between obese and non-obese. Differentiating metabolites were selected with the criteria of the variable importance in the projection (VIP) value>1 and *p* value (Student’s *t* test) lower than 0.05. Twenty metabolites were identified using this criteria, all of which were upregulated in the ovarian tumors of the non-obese versus obese KpB mice (Table 2).

Metabolites involved in inflammatory signaling and protein/collagen metabolism were down-regulated in the ovarian tumors of obese mice as compared to non-obese mice, including arginine (p=0.0268), N-glycylproline (p=0.0043) and 3-amino-2-piperidone (p=0.0099). Components and markers of oxidative stress were also downregulated in the tumors from obese mice: glutathione (p=0.0313), oxidized glutathione (p=0.0047), gluconolactone (p=0.0311) and 8-hydroxy-deoxyguanosine (p=0.0230). Lower levels of nucelotides (i.e. cytidine (p=0.0122 and p=0.0424), cytosine (p=0.0158), guanosine diphosphate (GDP, p=0.0404)) and adenosine monophosphate (AMP, p=0.0257) were detected with obesity. The serotonin metabolite, 5-hydroxyindoleacetic acid (5HIAA, p=0.0498), and the catecholamine metabolites, vanillactic acid (p=0.0079) and phenylethanolamine (p=0.0446), were found to be lower in the ovarian tumors of obese versus non-obese mice. Glutamate (p=.0318), N-acetylaspartic acid (p=0.0059) and succinic acid (p=0.0465) are

involved in energy metabolism, and were decreased in the ovarian tumors of obese KpB mice as compared to their non-obese counterparts. LysoPC(16:1(9Z)) (p=0.0205), a lysophospholipid, and the metabolite of a toxic intermediate, inodxyl glucuronide (p=0.0439), were also lower in the ovarian tumors from obese animals.

Table 2. Metabolic alterations in tumors from non-obese and obese KpB mice.

Compound name	VIP [a]	*p* [b]	Fold Change (non-obese/obese) [c]	Analysis method	Identification Method [e]
N-Glycylproline	2.27	0.0043	1.95	LC-ES+	Std
Oxidized glutathione	2.25	0.0047	3.45	LC-ES+	Std
N-Acetylaspartic acid	2.22	0.0059	2.31	LC-ES-	HMDB
Vanillactic acid	2.17	0.0079	2.23	LC-ES+	HMDB
3-amino-2-piperidone	2.14	0.0099	1.75	GCTOF	NIST
Cytidine	2.10	0.0122	4.52	LC-ES+	Std
Cytosine	2.05	0.0158	4.11	LC-ES+	Std
LysoPC(16:1(9Z))	1.99	0.0205	1.83	LC-ES+	HMDB
8-Hydroxy-deoxyguanosine	1.97	0.0230	2.45	LC-ES+	HMDB
Adenosine monophosphate	1.94	0.0257	1.61	LC-ES-	HMDB
Arginine	1.93	0.0268	1.93	LC-ES+	Std
Gluconolactone	1.89	0.0311	2.97	LC-ES+	Std
Glutathione	1.89	0.0313	3.10	LC-ES+	Std
Glutamate	1.89	0.0318	1.52	GCTOF	Std
Guanosine diphosphate	1.82	0.0404	2.39	LC-ES-	HMDB
Cytidine	1.81	0.0424	4.97	GCTOF	NIST
Inodxyl glucuronide	1.80	0.0439	3.05	LC-ES+	HMDB
Phenylethanolamine	1.80	0.0446	1.69	GCTOF	NIST
Succinic acid	1.78	0.0465	1.90	GCTOF	Std
5-Hydroxyindoleacetic acid	1.76	0.0498	1.85	LC-ES+	HMDB

[a] variable importance in the projection (VIP) was obtained from OPLS-DA with a threshold of 1.0; [b] *p* value was calculated from Student's *t* Test; [c] Fold change with a value larger than 1 indicates a relatively higher concentration in tumors from non-obese (low fat diet-fed) KpB mice, while a value less than 1 means a relatively lower concentration as compared to tumors from obese (high fat diet-fed) KpB mice. [e] The metabolites were identified by in-house library (Std), NIST library (NIST) or HMBD database (HMDB).

Slides have been made from these tumors and are undergoing immunohistochemical analysis to assess for differences in signaling targets of the PI3K/Akt/mTOR pathway. We have not found significant differences bet ween phosphorylation of mTOR or AMPK or the facilitative glucose transporter-1 (GLUT1), but we are also assessing other downstream targets of the metformin/mTOR pathway, including Akt, S6, 4EBP-1 as well as other GLUTs. These findings will be important for the cross-species evaluations between obese and non-obese mice and women in Task 3 (Aim 3).

Metformin inhibited tumor growth in the KpB mice fed a LFD and a HFD (n=8-10 animals per group), after one month of treatment (**Figure 8**). In the mice fed a HFD, metformin decreased tumor growth by 60% compared to control animals. Tumor growth was only decreased by 32% in the mice fed a LFD. A comparison of the anti-tumorigenic effects of metformin on mice fed a LFD versus a HFD demonstrated that metformin was more efficacious in mice on the HFD (p=0.003), suggesting that metformin may be more beneficial in the setting of obesity.

Tumor has been collected post-metformin treatment in the HFD and LFD groups and (1) will be submitted for metabolomic analysis, and (2) will undergo immunohistochemical/western blotting analysis to assess downstream targets of the mTOR pathway. RNA has been extracted from these tumors post-metformin

treatment and will be submitted for genomic analysis. Serum has been collected from these mice pre- and post-treatment for (1) measurement of glucose, leptin, insulin and adiponectin, and (2) metabolomic analysis. In the upcoming report period, these experiments will be replicated for everolimus in the KpB mice fed a HFD and LFD, and these mouse studies are currently underway.

Task 3 (Aim 3): Cross-species evaluation of differences between human and KpB mouse ovarian cancers in lean and obese states through genomics and metabolomics and primary culture.

We have collected ovarian tumors for primary culture from 10 patients to date. Unfortunately, 2 became contaminated, one was from a benign ovarian mass on final pathology and one was endometrioid instead of serous histology. Thus, we have only successfully assessed ovarian tumors from 6 patients (2 were of normal weight, 4 were overweight), and this data is too premature to report at this time. The tumors from these 6 patients were treated with metformin in short term primary culture, and all responded to this therapy. The effects of metformin on proliferation and apoptosis has been assessed as well as downstream targets of the metformin pathway by western immunoblotting analysis in the tumors from these 6 patients. We will continue to collect ovarian tumors and biofluids from obese and non-obese women in the upcoming report period.

KEY RESEARCH ACCOMPLISHMENTS

- Metformin and everolimus potentially drive glucose metabolism and uptake in OC cells, despite blunting proliferation.
- Metformin and everolimus inhibited proliferation in both cell lines under normal glucose conditions, through G1 cell cycle arrest.
- Metformin and everolimus were found to be more effective in the inhibition of proliferation under low versus high glucose concentrations, as also evidenced by enhanced G1 cell cycle arrest. In addition, metformin and everolimus were found to be more effective in the induction of apoptosis under low versus high glucose concentrations.
- As the concentration of glucose increased, phosphorylation of AMPK decreased and phosphorylation of S6 increased, suggesting increased proliferative capacity and hyperactivation of the mTOR pathway with high physiologic glucose
- The obese state can promote tumor progression in the KpB mouse model of ovarian cancer.
- Distinct metabolic and genomic differences were identified in ovarian tumors that arose in obese versus non-obese KpB mice, and many of these differences were related to metabolic relevant pathways.
- Metformin was more efficacious in our obese versus non-obese KpB mice.

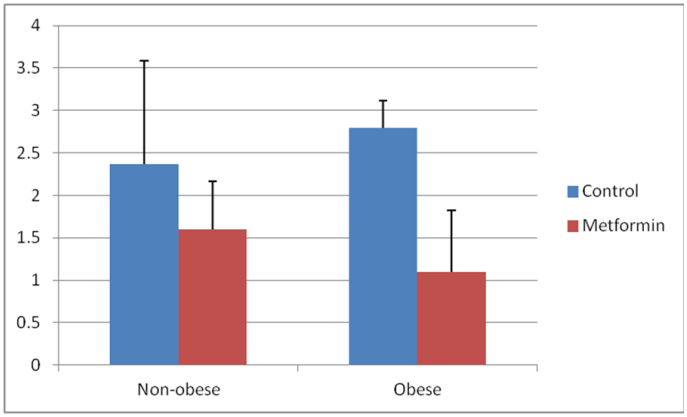

Figure 8. Metformin inhibited tumor growth in non-obese and obese KpB mice. In obese mice, metformin decreased tumor growth by 60% compared to obese control animals. Tumor growth was only decreased by 32% in the non-obese mice compared to non-obese controls. A comparison of the anti-tumorigenic effects of metformin on ovarian tumors from non-obese and obese mice demonstrated that metformin was more efficacious in obese mice (p=0.003), suggesting that metformin may be more beneficial in the setting of obesity.

REPORTABLE OUTCOMES

Abstracts presented:

(1) Zhou, C, Zhong, Y, Du, X, Makowski, L, Jia, W and Bae-Jump, VL, Diet-induced obesity increases tumor aggressiveness in a genetically engineered mouse model of serous ovarian cancer, Oral Presentation by Dr. Bae-Jump at the 2013 Annual Meeting of the Society of Gynecologic Oncology.

Manuscripts submitted:

(1) Makowski, L, Zhou, C, Zhong, Y, Kuan, PF, Fan, C, Sampey, BP, Difurio, M and Bae-Jump, VL., Obesity increases tumor aggressiveness in a genetically engineered mouse model of serous ovarian cancer, Manuscript submitted to the special issue of Gynecologic Oncology, "*The Obesity Crisis: Impact of Gynecologic Cancer*".

Grants submitted:

(1) 1R01CA185514-01 - RESEARCH ANSWERS TO NCIS PROVOCATIVE QUESTIONS- GROUP A (R01)
(PQA1) Obesity, Cation-Selective Transporters and Metformin in Endometrial Cancer
$1,000,000 (total direct costs), $250,000(annual direct cost for year one)
Co-Principal Investigator: Bae-Jump
20% effort (2.40 calendar)
Controversy surrounds whether metformin's anti-tumorigenic benefits stems from its indirect effects via decreasing circulating insulin and glucose levels or its direct effects in tumor cells via AMPK activation and inhibition of the mTOR pathway. Furthermore, it is unknown if metformin will be universally effective in cancer treatment or more efficacious in obese and insulin resistant patients, given its favorable impact on improvement in the metabolic syndrome. These fundamental questions will be explored in endometrial cancer, a disease driven by obesity and insulin resistance, using primary cultures of EC cells, EC mouse models and phase 0 and phase 2/3 clinical trials in EC patients.

CONCLUSION

Epithelial ovarian cancer is one of the most lethal cancers among women in the United States, and minimal improvements in overall survival have been made in the past several decades. The lack of progress is largely attributable to late detection, drug resistance, and a high recurrence rate. Although chemotherapeutic agents that target specific cell signaling pathways have greatly expanded our profile of ovarian cancer treatments, the challenge has been in identifying the patients that would most benefit from each of these diverse agents. To address these challenges, most previous research has focused on molecular alterations in the tumors derived from these patients. We postulate that focusing on the tumor alone may be too narrow a view and that the host environment, particularly the obese state, may play an equally important role in the selection of chemotherapeutic agents for effective treatment response. It is our hypothesis that obesity drives ovarian cancer formation through alterations in metabolic pathways; and thus, inhibitors of one such pathway (mTOR) may be more efficacious in the obese versus non-obese state.

In order to answer this fundamental biological question regarding the role of the obese environment in oncogenesis, our approach is three fold using ovarian cancer cell lines, a genetically engineered mouse model and patient samples. For each of these strategies, the metabolic state of obesity is being uniquely invoked and to test our hypothesis, two targeted mTOR pathway agents (metformin and everolimus) are being evaluated under obese versus non-obese conditions. To date, we have demonstrated that the obese state can promote tumor progression in the KpB mouse model of ovarian cancer, as evidenced by a doubling of tumor size in obese versus non-obese mice. Diet-induced obesity was mimicked in the KpB mice through exposure to a HFD. The ovarian tumors that arose in the obese mice were genomically and metabolically different from those that arose in non-obese mice. Metformin, an AMPK activator and mTOR inhibitor, was found to be more efficacious in the obese versus non-obese KpB mice, suggesting that obesity may be a biomarker for response to this agent. Studies in the upcoming report period will focus on evaluating the traditional mTOR inhibitor, everolimus, in obese and non-obese KpB mice.

For our *in vitro* studies, obesity/diabetes was mimicked by exposing ovarian cancer cell lines to high versus low and normal glucose conditions. We had hypothesized that metformin and everolimus would be more efficacious in the setting of high versus low and normal glucose, but we found the opposite to be true. Metformin and everolimus were found to be more effective in the inhibition of proliferation under low versus high glucose concentrations, as also evidenced by enhanced G1 cell cycle arrest. In addition, metformin and everolimus were found to be more effective in the induction of apoptosis under low versus high glucose concentrations. We postulate that ovarian cancer cells deprived of glucose may have blunted proliferative capacity, rendering them to be more susceptible to metformin and everolimus. In contrast, our studies indicate that a high glucose environment may enhance proliferative capacity. Further studies in the upcoming report period will focus on metabolic assays and protein expression to evaluate the full effects of metformin and

everolimus on glucose uptake and metabolism (glycolysis and oxidation) and downstream targets of the mTOR pathway under the varying glucose conditions.

We understand that the *in vitro* environment of high glucose exposure may not completely replicate that of the whole body *in vivo*; and thus, our animal and human studies are purposely meant to complement the cell culture work to better define the impact of obesity on ovarian cancer development, progression and ultimately, treatment. In order to explore the impact of obesity on sensitivity to mTOR inhibitor and metformin therapy in the human disease, primary cultures of freshly isolated human OC cells derived from obese versus lean patients are being exposed to these agents, and these results will eventually be correlated to those in our KpB mouse model. The human studies are underway, but the findings are too premature to report at this time.

Findings from this proposal should determine if obesity-driven ovarian cancers are biologically divergent and whether these inherent differences play a role in sensitivity to chemotherapeutic agents, and we have already provided evidence to support this hypothesis. This work may ultimately lead to the individualization of ovarian cancer treatment based on both tumor biology and the metabolic composition of the patient. This study will initially investigate this concept for the mTOR inhibitor everolimus and metformin in ovarian cancer, but may ultimately translate to other emerging therapies targeted to this pathway or others identified in this proposal. Future clinical trials of targeted therapies would have to be structured to address the host/tumor interaction, and we would propose that stratifying for obesity status may be an initial approach in this pursuit. To our knowledge, no cancer chemotherapeutic clinical trial has addressed obesity status as a contributing factor to therapeutic success. Lastly, this knowledge of the impact of obesity on response to targeted therapies would be important not just for ovarian cancer but for all cancers where obesity is associated with increased risk and worse outcomes, such as endometrial, breast and colon cancer among others.

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